PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: James A. Thomson | Date: September 22, 1997

Serial No.: 08/591,246 | Group Art Unit: 1815

Filed: January 18, 1996 | File No.: 960296.93723

For: PRIMATE EMBRYONIC STEM CELLS | Examiner: B. Brumback

DECLARATION UNDER 37 C.F.R. §1.131



Assistant Commissioner For Patents
Washington DC 20231

Dear Sir:

I, James A. Thomson, on oath say and declare that:

1. I am the same James A. Thomson who is the inventor of the above-identified patent application, and I make this declaration in support of that patent application.

2. I perform my research in the Wisconsin Regional Primate Research Center at the University of Wisconsin in Madison Wisconsin. I understand that one of the documents used by the patent Examiner to reject the claim of my patent application is a news report of my work carried in the Milwaukee Journal on November 4, 1994. That news report was the result of a press release issued by one of the administrators of the Wisconsin Regional Primate Research Center about the work which is reported in this patent application. Prior to November 1, 1994, I had created the

primate embryonic stem cell cultures identified as R278.5 and Cj11 as described in this patent application.

3. Attached to this declaration are two exhibits of records from my research. Exhibit A is a copy of a photograph of a karyotype of the embryonic stem cell culture designated R278.5. This karyotype was made and this photograph was taken prior to November 1, 1994. Attached hereto as Exhibit B is a copy of a laboratory notebook page from my laboratory notebook. The date on Exhibit B is redacted in the copy of Exhibit B, but the date on the original page is prior to November 1, 1994. The work reported on the laboratory notebook page which is Exhibit B is the successful culture of the cell line Cj11. At the bottom of Exhibit B, the notation "AP Rxn" refers to a successful test of this culture for alkaline phosphatase activity, one of the criteria recited in claim 3.

4. All the work described in this declaration or the Exhibits thereto was performed in the United States.

5. I hereby declare all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and the such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

James A. Thomson

QBMAD\142651

Exhibit A

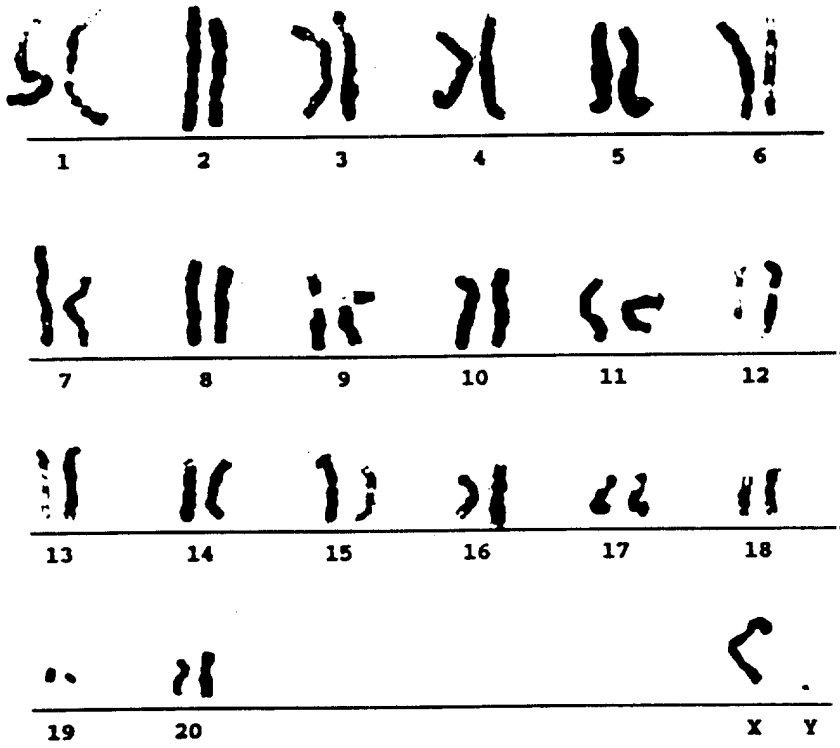


PRIMATE KARYOTYPE SHEET

SPECIES Macaca Mulatta. CASE NO. 378 Clone 5

INVESTIGATOR C. Harris PHOTO NO. 9-12-11

42, XY




Unpaired markers

ES: CJ-11 (from p 20)

EXHIBIT B 028

RECEIVED
OCT 1 5 1997

60169

1997

CJ 11.1 Good expansion of "flat" ES-like colonies; however endoderm differentiation taking place more abundantly. Only clone w/ good feeders (ie no over-growth)

CJ 11.2 Over-grown by feeders that were not properly inactivated. Very few ES-like colonies recognizable

CJ 11.3 Also contained w/ non-inactivated STO cells. Some very good [illegible] remain though.

CJ 11.4 Mostly endoderm; a few ES-like colonies.

① Added $Ca^{++}/Mg^{++}$ free PBS to each of above; choose individual colonies, trypsin 5' → chose individual cells → 96 well plates w/ 3T3 cells.
did 24 of 1 line [half has bFGF & h Steel factor (4 single)]
→ 5% $CO_2$ / 5% $O_2$
N:D manipulations took a long time & pH in media changed. (expect poor cloning efficiency if any)

② Split CJ 11.1 → T25 w/ 3T3 cells.

→ Will try to pan off fibroblasts/endoderm by allowing split & free cells to attach & keep floated after 15-30 minutes

4 x T75 = 2 T75
2 x T25 = 1.5 T25
[10 weeks]
[5 + 4]

① bFGF + h Steel factor 96 well plates: No colonies.
② ES 96 well plate ② colonies CJ 11.1, 3, 4
① colony CJ 11.2
Added new 3T3 cells w/o moving colonies.

③ Split CJ 11.1 → 2 T75's w/ 3T3 cells std 6/6/97
Some endoderm differentiation. (back in one flask)

④ Individual colonies w/o feeders of CJ 11.3 & CJ 11.4 still undifferentiated.

⑤ "Panning" did not work the 6 well plate of CJ 11.3 was very over-grown. However; Many good colonies remained: AP (Rex1 (Donovan, 1986) ④ ! ! ! ! ! !